



Thermal tolerance of the invasive red-bellied pacu and the risk of establishment in the United States

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ABSTRACT

Indigenous red-bellied pacu, *Piaractus brachypomus*, populations are in decline due to overfishing. Once ignored by aquaculturists because of their perceived low economic value, renewed aquaculture efforts in Central and South America aim to relieve fishing pressures on natural pacu populations. In the southern United States pacu aquaculture for the aquarium trade has raised concerns that accidental release could lead to establishment of overwintering populations outside captivity—a threat accentuated by the average 6 °C increase in shallow-water temperatures predicted by the end of the century. In the present study, Critical and Chronic Thermal Methodology was used to quantify red-bellied pacu thermal tolerance niche requirements. The data suggest that red-bellied pacu are a thermophilic species capable of tolerating low and high chronic temperatures of 16.5 °C and 35 °C, respectively. Critical thermal minimum and maximum temperatures of fish acclimated near their chronic limits are 10.3 and 44.4 °C. Red-bellied pacu aquaculture in the United States is concentrated in sub-tropical Florida regions that encourage rapid growth and reproduction, but carry an increased risk of establishing reproducing populations in local freshwater systems. The thermal niche data show that the risk of bioinvasion can be reduced or eliminated by adopting an approach whereby aquaculture potential is integrated with environmental temperature constraints.

1. Introduction

The combined effect of rapid global warming and geographic expansion of thermophilic invasive species has been defined as one of the most dangerous current threats to biodiversity (Di Santo, 2015; Hellmann et al., 2008; Rahel and Olden, 2008; Walsh et al., 2016). A warming climate will likely cause temperature increases in freshwater systems that could affect physiological performance, distribution, phenology and size of many aquatic species (Burrows et al., 2011; Di Santo, 2016; Di Santo and Lobel, 2017; Gardner et al., 2011; Genner et al., 2010; IPCC, 2013). In addition, anthropogenic activities have facilitated the spread of invasive fishes through implementation of fast-growing aquarium and aquaculture practices (Eme and Bennett, 2008; Rahel, 2007). Fish introductions into novel aquatic ecosystems can be devastating to indigenous diversity. While numerous adverse effects of exotic fish introductions have been explored such as disruption of native ecosystems (Dabruzzi et al., 2017) and increases in exotic diseases

or parasites (Daszak et al., 2000; Hoegh-Guldberg and Bruno, 2010; Marcogliese, 2001), the increased risk of survival and permanent establishment of non-native species in local ecosystems under global warming scenarios is currently understudied.

The recent increase in the number of exotic introductions in North American freshwater systems (Bajer et al., 2016; Larson et al., 2017) highlights a need for understanding the extent to which warming of freshwater bodies could provide suitable thermal conditions for survival and establishment of non-native fishes. Geographic regions supporting migration and establishment of thermophilic fishes are expected to increase as a consequence of warming (Bennett and Beitinge, 1997; Currie et al., 1998; McCauley and Beitinge, 1992; Semmens et al., 2004). For example, McCauley and Beitinge (1992) predicted that the ability to grow channel catfish, *Ictalurus punctatus*, in the United States would be extended northward by approximately 240 Km for each 1 °C increase in mean air temperature. It is likewise plausible that non-native aquaculture species could become a threat to local

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biodiversity as future temperature regimes become more amenable to colonization. Typically, fishes chosen for intense aquaculture production are hardy, tolerant species that thrive in marginal water conditions (e.g. low dissolved oxygen and high temperature), and therefore are less sensitive to changes in the environment when compared to wild indigenous fishes (Alcantara et al., 2003).

The red-bellied pacu, *Piaractus brachipomus* (Cuvier, 1818) is a Neotropical fish that has garnered some interest as an aquaculture species in the United States. Unlike their notorious relatives, the piranhas (genera *Serrasalmus*, *Pristobrycon*, *Pygocentrus*, and *Pygopristis*), with which they are often confused, pacu are fast growing frugivorous fish that tolerate marginal water quality (Lovshin et al., 1974) and prefer plant material to flesh. The species is a floodplain specialist distributed throughout the Amazon and Orinoco Rivers in the La Plata Basin of South America, and typically does not occur outside main floodplain channels (Correa et al., 2007). Red-bellied pacu have palatable flesh and are an important commercial species in their native habitat (Ferreira et al., 1996), but overfishing has resulted in population declines across their natural range. Once ignored by aquaculturists because of their perceived low economic value compared to the closely related tambaqui, *Colossoma macropomum* (Cuvier, 1816), renewed efforts to culture red-bellied pacu have been aimed at relieving overfishing pressures on natural populations (Alcantara et al., 2003; Avadí et al., 2014).

In the southern United States red-bellied pacu are cultured as an ornamental species for the aquarium trade (Chapman et al., 1997; Hill et al., 2009), with most exported fish cultured in the state of Florida (Chapman et al., 1997). While there are no indigenous pacu species in Florida, at least three species have been reported in state waters (Fig. 1), including red-bellied pacu, tambaqui, *Colossoma macropomum* (Cuvier, 1816), and caranha, *Piaractus mesopotamicus* (Holmberg, 1887). All three species are thought to have been introduced through aquarium releases or fish farm escapes. Major concerns over aquaculture of pacu in the United States are related to the risk of establishing permanent populations outside captivity. Within the U.S. there are frequent reports of pacu caught by anglers (Ćaleta et al., 2011), sometimes as far north as the Laurentian Great Lakes (Rixon et al., 2005). Low winter temperatures are believed to be the major factor preventing pacu from establishing permanent populations in the

northern United States, however; overwintering in thermal refugia such as stenothermal springs (see Edwards, 1977) or heated effluents (Neill and Magnuson, 1974) may allow pacu to survive winters in cooler northern regions. In their native tropical habitat, red-bellied pacu are found at temperatures between 15 and 35 °C (Lovshin et al., 1974), similar to conditions experienced in South Florida and the southernmost region of Texas (Bennett et al., 1997). The purpose of this study was to gain a better understanding of red-bellied pacu thermal ecology by quantifying the species' thermal niche using standardized thermal methodologies. Specific study goals were to 1) identify areas of the southern United States most susceptible to pacu colonization, 2) couple tolerance and local environmental data to identify sites within Florida suitable for pacu aquaculture but with minimal risk of bioinvasion, and 3) predict how future global warming scenarios may affect pacu populations in the United States.

2. Materials and methods

2.1. Red-bellied pacu holding conditions

One-hundred juvenile red-bellied pacu with an average standard length of 6.6 ± 1.46 cm, and mass of 12.1 ± 6.32 g, were purchased from a local distributor. Fish were transferred to the University of West Florida, Marine Eco-Physiology Facility where they were randomly sorted into five temperature treatment groups. Each temperature treatment was comprised of four replicate 35-L, glass aquaria containing five fish each, (i.e., 20 pacu per treatment). Fish were maintained in aerated, biologically filtered, de-chlorinated tap water (conductivity 220–350 $\mu\text{mho/cm}$; pH 7.2–7.6). Water quality including pH, ammonia, nitrite, and nitrate was tested in each aquarium twice per week and 20% water changes were performed weekly, or more frequently if indicated by water quality testing results. Pacu were fed TetraMin® conditioning food daily throughout the acclimation period but were not fed 24 h before or during trials.

2.1.1. Thermal acclimation protocols

All treatment group aquaria were housed in a Kysor, model PS-6 environmental chamber at an air temperature of 10 °C and a 12 h light: 12 h dark diel photoperiod. Techne®, model TE-10A, 1000-W, circulating heaters maintained individual aquaria within ± 0.5 °C of their temperature set point. Treatment aquaria water temperatures were initially set at 20.0 ± 0.5 °C, and adjusted to their final set-point temperatures once fish began actively feeding. Temperatures in two of the treatment groups were increased by 1 °C/day until reaching set-point temperatures of 25.0, and 30.0 °C. A third treatment group remained at 20.0 °C. Water temperatures in the two remaining treatment groups were increased or decreased by 0.5 °C per day until feeding cessation was observed. We used standard rates of increase and decrease in temperature that yield to meaningful prediction of thermal responses of organisms in the wild (Huey and Stevenson, 1979) and allow to compare thermal limits and niches across species. Treatment acclimation set-points of 16.5 and 35.0 °C (see Results) were then determined by reversing the direction of water temperature change in each treatment group until feeding resumed. Respective high and low feeding temperatures in each treatment were determined as the mean of the feeding cessation and feeding resumption points, and represent chronic maximum and chronic minimum temperatures that define the red-bellied pacu thermal acclimation range (Dabruzzi et al., 2017). All constant acclimation treatment groups were held at their final acclimation set-point temperatures for a minimum of 21 days before undergoing experimental trials.

2.2. Critical thermal methodology

Standard critical thermal methodology (CTM) was used to quantify critical thermal maximum (CTmax) and critical thermal minimum

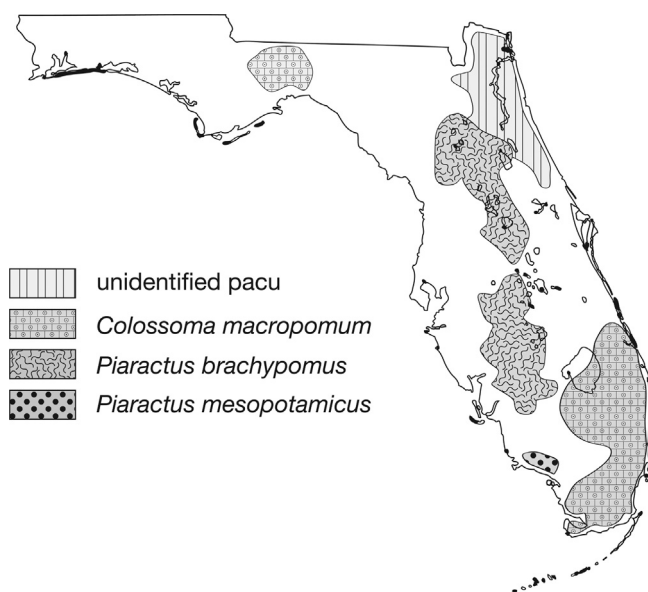


Fig. 1. Non-indigenous occurrence of different pacu species in Florida. According to the Florida Department of Environmental Protection pacu populations are thought to be introduced through aquarium releases or fish farm escapes and are listed as “permanent but not reproducing”.

(CTmin) temperatures of red-bellied pacu (Becker and Genoway, 1979; Beitinger and Bennett, 2000). In CTM trials, ten pacu from a given acclimation treatment were placed, one each, into 250- ml glass beakers filled with water from their acclimation aquarium. Beakers were placed into a re-circulating bath containing a 50:50 mixture of ethylene glycol and water. Moderate aeration in each beaker kept oxygen saturations high and prevented thermal stratification during trials. Beaker temperatures were indirectly increased (CTmax trials) or decreased (CTmin trials) by 0.2 °C/min, either by heating (Techne® TE-10A circulating heater), or cooling (MGW Lauda® model T-2 water chiller) the re-circulating bath mixture. Temperatures inside each beaker were monitored continuously with Traceable® NIST calibrated digital thermometers. While muscle spasms can be used as a CTM endpoint (e.g., Bonin et al., 1981; Paladino et al., 1980), final loss of equilibrium (LOE) was used in this study, as red-bellied pacu did not exhibit muscle spasms. Final loss of equilibrium was defined as the inability of the fish to maintain dorso-ventral orientation for at least one minute (Bennett and Beitinger, 1997). When LOE was observed, beaker water temperature was recorded, and the fish immediately removed from the beaker, massed (wet mass \pm 0.01 g), measured (standard length \pm 0.1 mm), and returned to its acclimation aquarium to recover. The CTmin and CTmax for each temperature treatment group was calculated as the arithmetic mean of the collective LOE replicate values.

Red-bellied pacu critical and chronic temperature tolerance data were used to define the species' ecological thermal niche, graphically expressed as a quadrilateral polygon (Bennett and Beitinger, 1997; Fangue and Bennett, 2003). The lateral polygon boundaries were determined by the lower and upper chronic tolerance limits. Simple linear regression lines of CTmax or CTmin on acclimation temperature were extrapolated to the upper and lower chronic temperature limits to define the upper and lower boundaries of the tolerance polygon. The resulting figure was expressed quantitatively using the areal units of °C².

Overwintering ranges and persistence zones for red-bellied pacu in Florida were determined by overlaying empirically derived cold tolerance data on mean January shallow water temperatures. The coldest air temperatures in the state occur during the month of January, and while shallow surface water temperatures are unavailable for much of Florida, they can be reliably predicted from mean air temperatures (Shafland and Foote, 1983; Shafland, 1979, 1986). Temperature-class limits used to construct map isotherms were determined using data obtained over a 30-year period by the National Oceanic and Atmospheric Administration, NOAA. Values were calculated separately by month (N = 30) and station (N = 46) so that on average, 33% of the observed mean January temperatures each would fall above, below, and within 1 °C of the isotherm value. Optimal temperature zones for red-bellied pacu growth in aquaculture applications were taken from estimations using genetic algorithm models (Bolte and Nath, 1996). Zones for optimal growth and assimilation were superimposed on overwintering estimations to determine ideal culture locations in Florida with minimal risk for establishment of permanent reproducing populations.

2.3. Statistical analyses

One-way analysis of variance (ANOVA) was used to test for significant effect of acclimation temperature on CTmin and CTmax. A Tukey-Kramer Multiple Comparisons Test (T-K MCT) was used to discriminate between means, and an analysis of covariance (ANCOVA) was performed using length or mass as covariates to assess the effects of these variables on temperature tolerance. The relationship between low temperature tolerance and acclimation temperature was quantified using regression analysis. All statistical analyses were performed in JMP version 12, and statistical decisions were based on an alpha of 0.05.

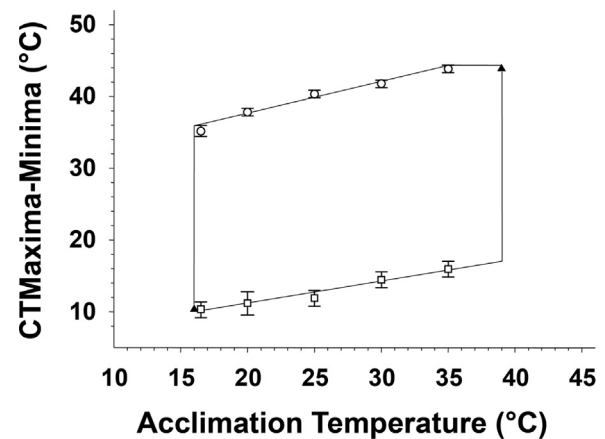


Fig. 2. Critical thermal minima (open square) and maxima (open circle) values for pacu acclimated to temperatures between 16.5 and 35 °C. Vertical bars represent 95% confidence intervals. Regression models of CTmax and CTmin on acclimation temperature were based on four replicates of five fish each at five constant temperature acclimation groups. Minimum and maximum chronic temperatures tolerated by pacu are reported (closed triangle).

3. Results

Fish body size, quantified as wet mass and standard length, had no apparent effect on red-bellied pacu chronic or critical thermal tolerance values. An analysis of covariance, using temperature tolerance as the response variable and mass or standard length as the covariate, found no significant differences between actual and predicted chronic or critical tolerance values (ANCOVA, all $p \geq 0.9$). All fish used in experimental heat or cold trials recovered quickly when returned to previous treatment temperatures, and resumed feeding 24 h post-trial. No pacu died during or subsequent to experimental trials.

Red-bellied pacu displayed a strong thermal tolerance response to decreasing acclimation temperature (Fig. 2). Mean critical thermal minima (\pm SD) of fish acclimated at constant treatment temperatures between their chronic thermal limits (16.5–35.0 °C) ranged from 10.3 ± 0.09 °C to 15.9 ± 0.06 °C, (Fig. 2). Values were significantly different (one-way ANOVA, $F=175$, $p < 0.0001$) and statistically distinct at each acclimation temperature (T-K MCT, $\alpha = 0.05$). Regression analysis found a significant relationship between CTmin and acclimation temperature ($r^2 = 0.943$, $p < 0.001$), expressed as $CTmin = 4.8 + 0.31 \times \text{acclimation temperature}$. The model indicates that pacu gain approximately 0.31 °C of cold tolerance for every 1 °C drop in acclimation temperature and that 94% of the variability in cold tolerance was explained by acclimation temperature alone.

Pacu exposed to increasing acclimation treatment temperatures also showed notable increases in heat tolerance. Fish acclimated at treatment temperatures between 16.5 and 35.0 °C had critical thermal maxima ranging from 35.14 ± 0.08 °C to 43.84 ± 0.06 °C. Mean CTmax values were significantly different (one-way ANOVA, $F=3160$, $p < 0.0001$) and statistically distinct at each acclimation treatment (T-K MCT, $\alpha = 0.05$). A significant linear relationship was seen between acclimation temperature and CTmaxima ($r^2 = 0.969$; $p < 0.001$), and was represented by the regression model, $CTmax = 28.9 + 0.43 \times \text{acclimation temperature}$. Approximately 97% of CTmax variability was explained by acclimation temperature. The acclimation response specified by the regression slope, suggests that pacu increase their heat tolerance by 0.43 °C with every 1 °C increase in ambient temperature.

The combined upper and lower chronic and critical limits for pacu produced an ecological thermal niche with a total thermal area of 635 °C² (Fig. 2). Reconciliation of Florida's mean January shallow surface water temperature data with pacu CTM data, suggests four potential overwintering outcomes for red-bellied pacu across the state

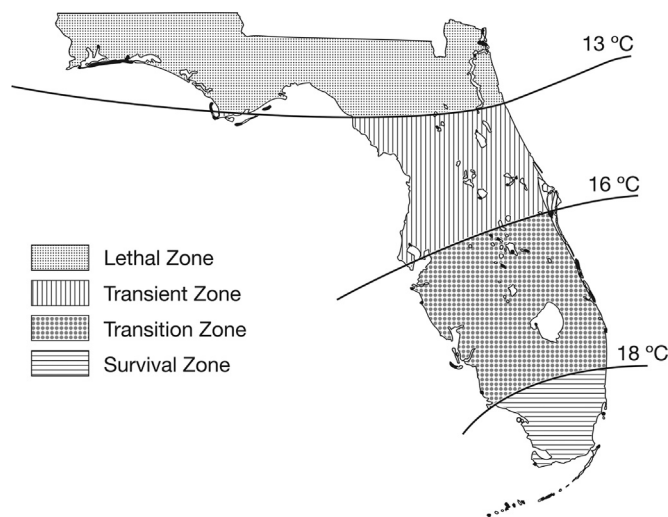


Fig. 3. Zonation of Florida into areas of different risks of introduction and establishment of red-bellied pacu populations. “Lethal” zone identify the area pacu would not be able to survive minimum winter temperatures; in the “transient” zone pacu could potentially overwinter in some years but the low temperature would maintain a low risk of establishment of permanent populations; in the “transition” zone, survival of fish is independent from temperature; the “survival” zone represents the area at risk of permanent establishment of red-bellied pacu.

(Fig. 3). The northern most “lethal-zone” is delimited by the 13 °C isotherm where winter temperatures routinely fall well below the red-bellied pacu low survival limit of ~16 °C. Pacu in this area would not survive exposure to low winter temperatures. Pacu could potentially overwinter, in the ‘transient-zone’ between the 13 and 16 °C January isotherms in a warm year, but the inevitable return of colder conditions would prevent establishment of permanent populations. January minimum temperatures between 16 and 18 °C encompass the “transition-zone” where temperatures may not be a direct threat to pacu survival, but populations could be limited by other factors such as competition, food availability, or adequate spawning habitat. The southernmost ‘survival’ zone is delineated by the 18 °C isotherm and represents the zone where pacu’s survival would be largely unaffected by temperature.

4. Discussion

The red-bellied pacu is a thermophilic fish species able to tolerate short-term exposure to high temperature extremes up to 44.4 °C. High tolerance values are not unexpected among Neotropical fishes of equatorial distribution but are unusual among North American species (Beitinger and Bennett, 2000; Currie et al., 1998). The thermal niche area for red-bellied pacu of 635 °C² is similar to other tropical species including the Indonesian sandflat goby *Bathygobius* sp. (Eme and Bennett, 2009) and the confamilial red-bellied piranha, *Pygocentrus nattereri* (Bennett et al., 1997). Overall, thermophilic fishes exhibit thermal niche area values between approximately 140 °C² for neon cleaner gobies (family Gobiidae; Di Santo and Lobel, 2017), to about 1500 °C² for pupfishes (family Cyprinodontidae; Bennett and Beitinger, 1997). Within this range the pacu thermal niche would rank as moderately large. Compared to more temperate fishes, the red-bellied pacu thermal niche is migrated upward in the direction of higher CT_{max} values, and to the right toward higher acclimation temperatures (Fig. 2). The shift in upper limits reflects a reduced dependence on cold-tolerance acclimation. Indeed, regression analysis confirms that for each 1 °C decrease in water temperature, pacu cold tolerance increases by only 0.31 °C, but the same temperature increase results in a 0.43 °C increase in heat tolerance, or a 40% greater acclimation gain. Overall, pacu accrue approximately 2 °C less cold tolerance than heat tolerance across their acclimation range, which can have implications for their

spread into non-native habitats. When mechanistic understanding of physiological responses, such as thermal tolerances are combined with local climatic data, it is possible to predict shifts in distributions, expansion and contraction of geographic ranges, and lay out the foundation for a strategic approach to develop synergies between empirical and theoretical data, management, and conservation of ecological units and communities.

The consequence of living at less than optimal thermal conditions is a key factor determining thermal vulnerability of fish populations (Beitinger and Bennett, 2000). Introduced pacu may for example experience impaired performance such as reduced feeding and/or loss of reproductive fitness due to the long-term effects of living at stressful temperatures (Bennett et al., 1997). Currently, no pacu populations are confirmed to be reproducing in the state of Florida although they are commonly found outside of captivity in high numbers (Fig. 1, Florida Fish and Wildlife Conservation Commission). The potential spread of pacu north of the 16 °C isotherm in Florida is likely controlled by cold temperatures. The lower tolerance limit for red-bellied pacu acclimated at 16.5 °C is 10.3 °C, but it is unlikely the fish would survive temperatures at or below 16.5 °C for more than several hours to a few days. Likewise, the inability to feed effectively at low temperatures suggests that pacu would do poorly between 16 and 18 °C. Although temperature in rivers and lakes can fluctuate 4–5 °C daily (Martin et al., 2006), our data suggest that pacu could survive during these thermal fluctuations, regardless of the presence of thermal refugia. High temperatures in south Florida should pose no direct threat to pacu survival, however Bolte and Nath (1996) report that breeding and growth of fish in shallow aquaculture ponds are generally impaired at water temperatures above 30 °C. It may be reasonable to assume, therefore, that pacu reproduction would be impaired in southern Florida zones where shallow water temperatures exceed 30 °C for extended periods in summer. Nevertheless, future studies could compare thermal tolerances and sensitivities of native and perhaps hardier, cultured fishes to quantify the risk of permanent establishment of individuals selected to survive in marginal conditions.

It is possible that red-bellied pacu could exploit physical as well as physiological conditions to support populations outside the 18 and 30 °C limits. For example, Mexican tetra, *Astyanax mexicanus*, released into Town Lake near Austin Texas, overwinter in a springhead nearly 600 km north of their previous northern most distribution limit (Edwards, 1977). Similarly, pacu released into watersheds originating at one of the more than 100 stenothermal springs stretching across the Florida peninsula (Zale, 1987) might mitigate adverse climate effects and optimize metabolic performance by exploiting spring thermal heterogeneity (Di Santo and Bennett, 2011a, b; Papastamatiou et al., 2015; Sims et al., 2006). In addition, smaller fishes may perform better in warmer environments (Baudron et al., 2014; Di Santo, 2016; Di Santo and Lobel, 2016; Gardner et al., 2011; Genner et al., 2010) and are less vulnerable to thermal challenges than larger and older conspecifics (Clark et al., 2017; Di Santo and Lobel, 2017). While pacu thermal tolerance was not significantly affected by fish mass or length in this study, only a relatively small size range of fish was used. If smaller pacu do in fact have higher survival rates during south Florida’s hot summer months, they would comprise the majority of breeding cohort perpetuating the population into the next season.

Introduction of alien species and their negative impacts on the local ecosystems has become one of global importance, particularly as biological control measures to eradicate already established exotic species (e.g., predation, competition, parasite and pathogen infestation) is still problematic (García-Berthou, 2007). Consequently, there is an urgent need to develop management strategies that mitigate or prevent bioinvasions. The southeast United States, and Florida in particular, is a known hotspot of exotic fish invasion, and this is a serious threat to the area’s already fragile freshwater ecosystems (Eme and Bennett, 2008). The import and accidental release of thermophilic species into novel areas that would allow the permanent establishment is already a

widespread issue that has imminent and long-lasting consequences for conservation and management of native species (Eme and Bennett, 2008; Semmens et al., 2004). Currently, the demand for Florida-raised pacu is largely limited to the pet industry although some potential exists for future cultivation as food species. Pacu are already found outside captivity in Florida and many other states, and repeated frequent releases of this species into Florida habitats raises the probability that the fish may have already established permanent populations in the United States (Semmens et al., 2004). The potential for establishment of pacu might be effectively mitigated by limiting aquaculture of the fish to areas that are too cold during the winter to support them in the north and too warm to allow effective growth and reproduction in the south. At present, this zone would likely exist between Gainesville and Tampa (Fig. 3). Currently, most culture facilities are located in thermal zones amenable to fast growth and reproduction of the species, conditions that may also increase potential of exotic introduction and thriving reproducing populations. Projected temperature increases from climate change are being revised each year (Hellmann et al., 2008; IPCC, 2013; Rahel and Olden, 2008) making it is difficult to identify areas in Florida, and other southern states, where red-bellied pacu could be cultured with no future risk for exotic introduction. Even so, an approach whereby culture potential is closely integrated with environmental constraints may reduce threats of introductions in the future.

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